Strawberry Necrotic Shock Virus: A New Virus Previously Thought to Be *Tobacco Streak Virus*

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Keywords: *Fragaria × ananassa, Strawberry necrotic shock virus, Tobacco streak virus,* detection

Abstract

***Tobacco streak virus* (TSV) has a wide host range that exceeds 80 species (Fulton, 1948). Most of the efforts carried out previously comparing TSV isolates was based on immunological relations between them. The isolates of the virus from *Fragaria* and *Rubus* have been considered to be very closely related if not identical while they are distinct from isolates of other hosts. While trying to characterize the viruses associated with pallidosis disease, we cloned part of the genome of a virus with homology to TSV but distinct enough to be considered a unique virus and not an isolate of TSV. Here we report the complete sequence of RNA 3 of the virus from a strawberry isolate as well as the sequence of the coat protein gene from an additional nine strawberry as well as five *Rubus* isolates. The data suggest that *Fragaria* and *Rubus* are infected with a virus closely related to TSV, designated as *Strawberry necrotic shock virus* from the name given by Frasier et al. in the first report of the virus infecting strawberry. We were unable to acquire any amplicons utitizing reverse transcription-polymerase chain reaction with primers derived from the published sequence for TSV, an indication that strawberry and *Rubus* may not be hosts for TSV.**

INTRODUCTION

Tobacco streak virus (TSV) is one of the more than 30 virus and virus-like agents infecting strawberry (Spiegel and Martin 1998). TSV is the type member of the *Ilarvirus* group of the family *Bromoviridae*. Viruses belonging to the family have tripartite genomes that are encapsidated in quasi-isometric particles of 27-35 nm. RNA 1 encodes the viral replicase that has methyltransferase and helicase motifs and in the case of *Brome mosaic virus* is involved in the anchoring of the replication complex in the membranes (den Boon et al., 2001). RNA 2 encodes the viral polymerase and some members of the family also encode a protein that may function as a suppressor of RNA interference (gene silencing) (Xin et al., 1998). RNA 3 encodes the movement and coat protein of the virus. The coat protein is expressed from a subgenomic RNA (RNA 4) and is needed for long distance movement and genome activation (Jaspars, 1999). Ilarviruses are pollen and seed transmitted and thrips transmission has been demonstrated for several members of the genus (Sdoodee and Teakle 1987).

TSV in small fruits was documented first in the 1960's (Frazier et al., 1962) when it was thought to be a new virus species and given the name *Strawberry necrotic shock virus* (SNSV). *Black raspberry latent virus* (BRLV) also was originally thought to be a distinct virus (Converse and Lister 1969). Previous studies with TSV in small fruits were based on symptomatology on indicator plants, serological relationships and/or hybridization studies. Several of the previous studies demonstrated differences between small fruit strains and other strains of TSV. Small fruit strains of TSV were not able to cross-protect plants when challenged with other strains (Fulton, 1978). Stenger et al. (1987) performed Northern blot analysis and where unable to detect the *Rubus* and strawberry isolates of the virus when using probes developed from the white clover (WC) strain of the virus and vice versa.

While characterizing the viruses that are involved in pallidosis disease we acquired clones that were related at the amino acid level with TSV but the nucleotide homology was minimal for suggesting that these clones represented a virus distinct from TSV. All attempts to acquire amplicons utilizing oligonucleotide primers developed from published sequences of TSV for detection of the virus in small fruits were unsuccessful. Given the uniqueness of the sequence obtained, the cloning and sequencing of RNA 3 of a strawberry isolate of the virus and the coat protein gene of several isolates from strawberry and *Rubus* was carried out. Here the results of these studies are presented, indicating that what was considered small fruit isolates of TSV is a unique virus, designated as *Strawberry necrotic shock virus.*

MATERIALS AND METHODS

Small fruit isolates used in the study were acquired from the National Clonal Germplasm Repository in Corvallis, Oregon (Table 1.) except the red raspberry isolate that belongs to our virus collection and a strawberry field isolate from Maryland, USA that was used to determine the complete sequence of RNA 3. TSV isolates of herbaceous hosts used in the study were in the form of dessicated tissue (Table 1.).

The sequences of the study were obtained utilizing dsRNA purified as described previously (Yoshikawa and Converse, 1990) or ssRNA extracted according to the Hughes and Galau method (1987) as templates for random cloning or reverse transcription-polymerase chain reaction (RT-PCR). All reactions were carried out utilizing products of Invitrogen Corp. (Carlsbad, CA). All primers utilized for the PCR are listed in table 2. The consensus sequence of each individual isolate was determined from at least two clones derived from two individual PCRs that were performed utilizing the Platimum ® Taq polymerase and the sequence obtained directly from the PCR products. Alignments were performed with CLUSTAL W at http://www.ebi.ac.uk/clustalw (European Bioinformatics Institute). Detection of the viruses utilizing RT-PCR with Superscript III® and Taq polymerase was performed utilizing primers SNSV CPbegF/SNSV CPendR and TSV CPF/ TSV CPR utilizing a PCR program that consisted of five minute denaturation step at 94°C followed by 40 cycles of 30 sec at 94°C, 30 sec at 50°C and 1.5 min at 72°C. The program concluded with a final extension step for 10 min at 72°C. Amplification of the 3' end of RNA 3 was performed after adenylation of the molecule (Sippel, 1973) utilizing SNSV CPendF and Adapter primers. The 5'end of the virus was determined after performing 5'RACE with primers SNSV MPbeg R and AAP. Phylogenetic analysis was performed with the GCG Wisconsin Package utilizing the Jukes-Cantor distance correction and the UPGMA tree construction methods at default settings.

RESULTS AND DISCUSSION

The complete nucleotide sequence of RNA 3 of a strawberry isolate from Maryland, USA of SNSV was determined. The molecule is 2248 nucleotides, 43 nucleotides longer than that of the white clover isolate of TSV (TSV-WC, GenBank accession No NC 003845), that has been sequenced previously. The overall nucleotide identity between the two viruses was 70%. The molecule has two open reading frames (ORFs). The 5' proximal ORF encodes the viral movement protein that is 298 amino acids long, 9 amino acids longer than that of TSV-WC. The overall amino acids identities with TSV-WC is 74% and 63% with *Parietaria mottle virus* (PMoV) (GenBank Accession number U35145). The coat protein gene (CP) of SNSV encodes for 222 amino acids in contrast to the 238 amino acids encoded by the TSV CP. The overall amino acid identities between the 15 isolates of SNSV CP sequenced is 60-65% with TSV-WC and 42-43% with PMoV.

Oligonucleotide primers designed for detection of SNSV were used successfully to detect all isolates of SNSV (TSV isolates from *Fragaria* and *Rubus*) of the study while no amplicons were obtained with the TSV isolates used as controls. Conversely, amplicons were obtained with all three isolates of TSV from other hosts when TSV specific primers were used but not with any of the SNSV isolates. These products were

sequenced and had from 99.7-100% nucleotide identities with the sequence of TSV-WC.

The phylogenetic analysis (Fig. 1) performed revealed clusters of the *Fragaria* and *Rubus* isolates with the exception of the field strawberry isolate used to determine the sequence of RNA 3 that clustered with the *Rubus* isolates. This discrepancy may be attributed to either the geographic origin of the isolates or alternatively to the inter-species transmission of the virus with a thrips vector (Sdoodee and Teakle, 1993). All *Rubus* isolates originated in the Pacific Northwest region of the United States in contrast with the Maryland strawberry isolate from the mid-Atlantic coast. It is unlikely that these isolates are related closely because of the geographic origin, especially when two strawberry isolates that originated in North Carolina (1314) and California (9078) were identical. It is interesting that the three Japanese isolates used in the study (1268, 1291, 1294) cluster together but the small number of sampling does not allow to draw any definite conclusions from that observation.

It has also been concluded that BRLV, previously thought to be a strain of TSV, and a quarantine pathogen for some countries, is a strain of SNSV and can be readily assayed by RT-PCR or ELISA using tools designed for SNSV detection.

SNSV causes losses in strawberry production and can have synergistic effects when in occurs in mixed infections with other viruses (Stace-Smith et al., 1987). Yield can be reduced up to 15% and runner production up to 75% (Johnson et al., 1984). Seed transmission of SNSV can reach up to 25% in black raspberry (Johnson et al. 1984) and 35% in strawberry (Converse 1979) making it a concern for *Fragaria* and *Rubus* breeding programs. The molecular data presented here allows for the sensitive and reliable detection using RT-PCR, an essential tool for elimination of viruses in nursery production systems. Future plans include studying cranberries infected with TSV (Jones et al., 2001) and investigating the possibility of cranberry being another small fruit host for SNSV.

ACKNOWLEDGEMENTS

We would like to thank Joseph Postman at the NCGR, Corvallis, OR for providing the *Fragaria* and *Rubus* SNSV isolates used in the study.

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Tables

Table 1. Plant species, Cultivar Name/Isolate, NCGR identification number and region of origin of the isolates used in the study.

Plant species	Cultivar, Isolate / NCGR No.	Region
Strawberry	US-70 / 1189	Mississippi
Strawberry	US-159 / 1191	Mississippi
Strawberry	Melcher / 1196	Louisiana
Strawberry	Koro / 1268	Japan
Strawberry	Ooishi-shikinari / 1291	Japan
Strawberry	Sokusei / 1294	Japan
Strawberry	Sweet Charlie / 1314	North Carolina
Strawberry	G980 / 9034	California
Strawberry	MS-US-540 / 9078	North Carolina
Strawberry	Field isolate (MD)	Maryland
Blackberry	Dyke / 139	PNW*
Black Raspberry	New Logan (BRLV) / 9012	PNW
Wild Blackberry	*Rubus ursinus* / 9025	PNW
Red Raspberry	N/A (RR)	PNW
Black Raspberry	Munger / 9003	PNW
Bean**	White clover (WC)	N/A
Tobacco**	Tobacco isolate	N/A
Bean**	N/A	N/A

PNW* = Pacific Northwest
** = TSV isolates

Table 2. Oligonucleotide sequence used for detection of SNSV and TSV and determination of the sequence of RNA 3 of SNSV.

Primer Name	Nucleotide sequence (5'-3')
AAP	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG
SNSV MPbeg R	CAGTGTTTACGGCTGCGAAG
SNSV MPbeg F	GGGATCGATTGGTTAGGACCGTCAT
SNSV CPbeg R	ACACCACCATTGCGCATACATCTC
SNSV CPbeg F	GAGTATTTCTGTAGTGAATTCTTGGA
SNSV CPend R	ATTATTCTTAATGTGAGGCAACTCG
SNSV CPend F	GACCTAATCCGTTGATGCCTCCAGA
Adapter Primer	GGCCA CGCGT CGACT AGTAC$(T)_{18}$
TSV CP F	ACGAGTATTAAGTGGATGAATTCT
TSV CP R	ACTTACAATACGTCGAGGTGTG

Figures

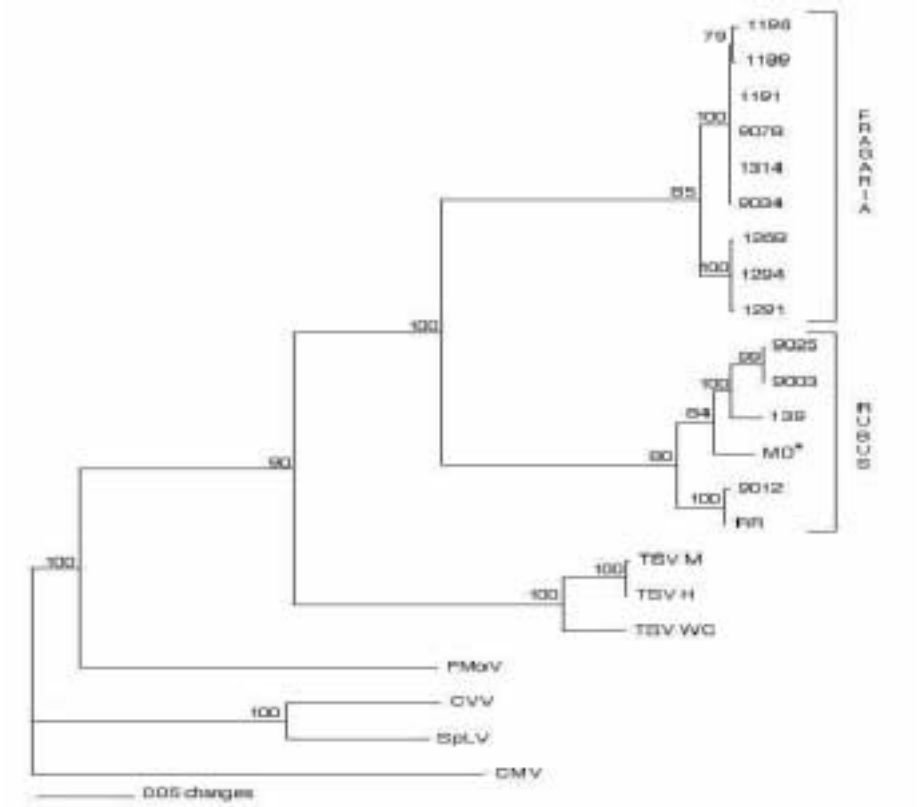

Fig. 1. Phylogram of the coat protein gene of 15 *Strawberry necrotic shock virus* isolates and other Bromoviruses. The strawberry and *Rubus* isolates are identified in Table 1. Abbreviations and GenBank Accession numbers of the other viruses: TSV-WC, *Tobacco steak virus* (TSV) white clover isolate, NC 003845; TSV-M, TSV Mungbean isolate, AF 515823; TSV-H, TSV Sunn-hemp isolate, AF515825; PMoV, *Parietaria mottle virus,* U 35145; CVV - *Citrus variegation virus,* AF 434912; SpLV, *Spinach latent virus,* NC 003810; CMV, *Cucumber mosaic virus*, AF 523352. The asterisk indicates the strawberry field isolate from Maryland, USA, that clusters with the *Rubus* isolates. The bootstrap values are given in percentage values. The bar is normalized to represent 0.05 nucleotide changes per site. CMV is used as the outgroup.